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Research paper

Evaluation of the chemical composition of a mixture of sugarcane bagasse and straw after different pretreatments and their effects on commercial enzyme combinations for the production of fermentable sugars

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ABSTRACT

Replacement of fossil fuels with renewable sources has been studied as a strategy to mitigate the environmental impacts of energy production. These concerns have encouraged the use of alternative sources of cost-competitive and sustainable biofuels, such as lignocellulosic biomass, for second-generation ethanol production. As the enzymes required for biomass hydrolysis are costly, the development of less expensive enzyme mixtures for the hydrolysis of sugarcane biomass is important. The efficient conversion of biomass to fermentable sugars requires a critical pretreatment step to increase enzyme accessibility to the substrates during hydrolysis. Ionic liquids (ILs) have recently emerged as interesting solvents for biomass pretreatment. Extensive research has been conducted, showing that IL pretreatment can increase fermentable sugar productivity and biomass hydrolysis yields in comparison with conventional pretreatments, such as dilute sulfuric acid (DSA). The objective of this work was to evaluate changes in the chemical composition of a sugarcane biomass mixture composed of straw and bagasse in a 1:1 (w/w) ratio after pretreatment with the IL 1-ethyl-3-methylimidazolium acetate ([Emim][Ac]) or with diluted sulfuric acid and determine the effect of the pretreatments on the interaction between commercial enzymes for the production of fermentable sugars by enzymatic hydrolysis. Pretreatments with IL were more efficient in the removal of lignin than pretreatments with DSA. Cellulase and endo-1,4-xylanase had a higher contribution to the release of glucose and xylose for both pretreated samples. The highest values of glucose and xylose yields, 99.96% and 86.52%, respectively, were achieved with the enzymatic hydrolysis of the biomass pretreated with IL.

1. Introduction

The scarcity of fossil fuel reserves, coupled with environmental damages, has led to a growing need for increased production and consumption of fuels that are renewable and sustainable. Ethanol is a liquid biofuel with the potential to replace partially the gasoline needed for transportation worldwide. Presently, ethanol is mainly produced from sugarcane juice (sucrose) in Brazil [1] and from corn (starch) in the USA [2]. However, it is well established that widespread use of ethanol will require new sources of raw materials that have the advantage of being widely available, such as wood and agricultural residues [3]. Even so, the cost of ethanol production from lignocellulosic biomass with current technologies is still very high [4].

These concerns have encouraged the exploration of cost-competitive and sustainable supplies of biofuel [5]. Sugarcane bagasse and straw have attracted the interest of scientists in Brazil as potential sources for second-generation ethanol production [4], which can be explained not only by their chemical composition rich in polysaccharides, such as cellulose and hemicellulose, but also by the proximity great availability of these by-products near ethanol production mills and the consequent reduction in transport costs [6].

The production of ethanol from lignocellulosic materials requires four main steps: pretreatment of biomass, saccharification, fermentation, and distillation [7]. The lignocellulosic material must be pretreated to allow a higher conversion of cellulose and hemicellulose, which are consumed during enzymatic hydrolysis to produce glucose and xylose, sugars that are then fermented to produce ethanol [8]. Pretreatments act by disrupting the lignocellulosic matrix, reducing the amount of lignin and hemicellulose, and modifying the crystalline structure of cellulose to make it more susceptible to enzymatic attack [9]. The yield of conversion of lignocellulosic materials into glucose and xylose is usually very low if the enzymatic hydrolysis is carried out without a pretreatment. Thus, pretreatments are applied when a high yield of conversion into glucose and xylose are required after the

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enzymatic hydrolysis of the solid fraction of the biomass [9,10].

Many different strategies have been developed to convert polysaccharides into fermentable sugars. One strategy involves the conventional pretreatment with dilute acids, which promote intense hemicellulose solubilization at high temperatures [11]. On the other hand, the utilization of such chemicals requires the use of acid-resistant equipment parts and a neutralization step for treating the effluent. Furthermore, saccharification and fermentation inhibitors are formed when the biomass is pretreated with acids [12].

Recently, the use of ionic liquids (ILs) was shown to be efficient in the pretreatment of biomass, as they were able to reduce the crystallinity of cellulose and partially remove hemicellulose and lignin while not generating degradation products that are inhibitory to enzymes and fermenting microorganisms [13]. ILs have low volatility and are non-flammable and easily recyclable [14]. Recent advances have been made in biomass pretreatment and production of fermentable sugars, especially in regard to new fractionation methods that use [Emim][Ac] as a pretreatment agent (as presented in the studies of da Costa Lopes et al. [15] and Da Silva et al. [16]). These new methods allow the separation of high-purity cellulose, hemicellulose, and lignin fractions as well as a high IL recovery.

The lignocellulosic industry of the future will likely use many different pretreatment/biomass combinations, and therefore enzyme mixtures that can handle different pretreatment/biomass combinations will be necessary [17]. Although there are a considerable number of studies on sugarcane bagasse as biomass, there is a lack of studies on sugarcane straw and even less that use a mixture of bagasse and straw as substrate for the production of fermentable sugars. The development of more efficient lignocellulose-degrading enzyme cocktails will require deeper and more precise knowledge on the specific enzymes that are involved in the hydrolysis of sugarcane biomass [18].

In the present work, we report the study and quantification of changes in the chemical composition of a mixture of the two main lignocellulosic fractions of sugarcane biomass, bagasse and straw, in a 1:1 (w/w) ratio. Changes in the chemical composition were caused by the different performance of the pretreatments applied, using dilute sulfuric acid (DSA) or the ionic liquid 1-ethyl-3-methylimidazolium acetate ([Emim][Ac]) under mild conditions. These conditions were employed to disorganize the structure of the lignocellulosic biomass and, at the same time, to preserve the sources of hemicellulose. The second step of this work was an exploratory study aimed at investigating the interaction of five different commercial enzymes using a Plackett–Burman design. Experiments were designed to allow the evaluation of simultaneous production of xylose and glucose and compare the differences obtained when using biomass pretreated with the commercial IL or biomass pretreated by a conventional method (using dilute sulfuric acid). Pretreatment conditions and the association of their effects with the action of different enzyme mixtures on biomass hydrolysis, as well as the type of biomass used, make this study relevant and original. Although there are many reports in the literature on the use of sugarcane bagasse or straw as biomass, there is a lack of information about the use of these two fractions together, about mild pretreatment conditions with ILs compared with dilute sulfuric acid, and about differences in the effects of cellulase and hemicellulase mixtures in response to the pretreatments. Therefore, this study aimed to fill this research gap. [Emim][Ac] was chosen because of its low melting temperature, low viscosity, non-toxicity, and non-corrosivity, which are advantageous characteristics when compared to those of sulfuric acid, a toxic and corrosive solvent [19]. Although [Emim][Ac] has a high cost, there are reports in the literature of biomass pretreatments using low-cost ILs (approximately US$ 1/kg) [20]. Thus, it is important to emphasize that [Emim][Ac] was used as a proof of concept to compare an IL pretreatment with dilute sulfuric acid pretreatment for subsequent application of enzyme mixtures.

2. Materials and methods

2.1. Sugarcane biomass

Sugarcane bagasse and straw were kindly supplied by Raízen, located in Piracicaba, São Paulo, Brazil, and by Dr. Paulo Graciano, from the Brazilian Bioethanol Science and Technology Laboratory (CTBE/CNPEM), Campinas, São Paulo. The moisture content of the raw materials was quantified according to the AOAC method [21]. Sugarcane bagasse and straw contained 45% and 48% moisture (wet base), respectively. Both sugarcane biomass fractions were washed with water at room temperature to remove the excess of soil and dust [22]. Samples were oven-dried at 80 °C to at least 10% moisture (dry base), then ground in a cutting mill and sieved to retain particles in the range of 24–48 mesh. Sample humidity was kept at about 8–10% (dry base) for subsequent chemical characterization and application of pretreatments.

2.2. Dilute sulfuric acid pretreatment

A 1:1 (w/w) mixture of bagasse and straw was pretreated with two different concentrations of sulfuric acid, 0.5% (w/v) and 1% (w/v). The solid loading (5 wt %), temperature (100 °C), and residence time (30 min) were the same for the two experimental conditions. Initially, the biomass and the aqueous acid solution were added to a 250 mL Erlenmeyer flask immersed in a water bath with electrical heating. The residence time count initiated when the temperature reached the programmed value. After 30 min, the pretreatment was interrupted by immersing the flask in an ice bath, quenching the reaction. Then, the hemicellulose hydrolysates were separated from the solid fraction by vacuum filtration. The remaining solids were thoroughly washed with deionized water, oven-dried overnight at 105 °C, and stored in sealed bags at room temperature.

2.3. IL pretreatment

A 1:1 (w/w) mixture of bagasse and straw was pretreated with the IL [Emim][Ac], purchased from (Iolitec-Germany) in two experimental conditions. The first pretreatment condition was carried out at 100 °C using a thermostated bath (TB). As an alternative source of energy, the second pretreatment condition was performed at 25 °C using an ultrasonic bath (UB) at 25 kHz, 160 W, and 25 °C. The solid loading (5 wt %) and residence time (30 min) were the same for the two experimental conditions. Subsequently, deionized water was added to the IL solution in a 5:1 (v/w) ratio to recover the biomass [23]. Then, the solids were washed repeatedly with deionized water until the wash solution was colorless to remove IL from the samples [24]. After the experiments, the IL/water mixture and the solid fraction were separated by vacuum filtration. The remaining solids were collected, oven-dried overnight at 105 °C, and stored in sealed bags at room temperature.

2.4. Efficiency of pretreatments

The amount of solid fraction of the pretreated biomass was quantified after each pretreatment process using Equations (1) and (2). This value is necessary to evaluate the pretreatment process in relation to the reduction of lignin and hemicellulose [10].

$$\text{Solid fraction}\left(\frac{\text{g}}{\text{g raw material}}\right)$$

$$\%\ \text{Recovered mass} = \left(\frac{\text{Final amount of insoluble material}}{\text{Initial amount of raw material}}\right) \times 100 \quad (1)$$

$$\%\ \text{Solubilization mass} = \left\{1 - \left(\frac{\text{Final amount of insoluble material}}{\text{Initial amount of raw material}}\right)\right\} \times 100 \quad (2)$$